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Review: Modelling placental amino acid transfer – From transporters to placental function

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ABSTRACT

Amino acid transfer to the fetus is dependent on several different factors. While these factors can be understood in isolation, it is still not possible to predict the function of the system as a whole. In order to do this an integrated approach is required which incorporates the interactions between the different determinants of amino acid transfer. Computational modelling of amino acid transfer in the term human placenta provides a mechanism by which this integrated approach can be delivered. Such a model would be invaluable for understanding amino acid transfer in both normal and pathological pregnancies.

In order to develop a computational model it is necessary to determine all the biological factors which are important contributors to net amino acid transfer and the ways in which they interact. For instance, how different classes of amino acid transporter must interact to transfer amino acids across the placenta. Mathematically, the kinetics of each type of transporter can be represented by separate equations that describe their transfer rate as a non-linear function of amino acid concentrations. These equations can then be combined in the model to predict the overall system behaviour. Testing these predictions experimentally will demonstrate the strengths and weaknesses of the model, which can then be refined with increasing complexity and retested in an iterative fashion.

In this way we hope to develop a functional computational model which will allow exploration of the factors that determine amino acid transfer across the placenta. This model may also allow the development of strategies to optimise placental transfer in pathologies associated with impaired amino acid transfer such as fetal growth restriction.

1. Introduction

Placental amino acid transfer is essential for fetal growth. In growth restricted fetuses amino acid transfer across the placenta is reduced and in animal models amino acid transfer has been shown to be decreased prior to the onset of fetal growth restriction, emphasising its causal role [1,2]. It is therefore important to understand the factors which determine amino acid transfer across the placenta if we are to develop interventions and preventive strategies to optimise fetal growth.

Placental amino acid transfer is dependent on multiple factors including: amino acid transporter characteristics, flow and mixing of maternal and fetal blood, placental structure and the levels of amino acids within the maternal, fetal and syncytiotrophoblast compartments. However, the complex interactions between these factors mean that the effect of all these determinants together cannot be intuitively predicted [3]. This limits our ability to understand how these factors contribute to net flux across the placenta and which of these factors are most likely to be rate-limiting for amino acid transfer and so for fetal growth. Here we overview the concept of computational modelling as one way of meeting this challenge.

This review will begin by outlining our approach to developing a computational model. It will then consider the processes by which amino acids are transported across the placenta in order to identify those factors which are likely to be important components of a computational model. Finally, our modelling approach will be discussed and its potential importance in understanding fetal growth.

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2. Modelling placental amino acid transfer

The aim of modelling placental amino acid transfer is to be able to predict how changes in specific placental parameters affect transfer of amino acids. To do this without becoming unnecessarily complicated, the model must focus on the factors that are the principle determinants of amino acid transfer in both normal and pathological pregnancies. Factors which are necessary for amino acid transfer, but which do not in practice become rate limiting, do not need to be modelled. As it may not be immediately apparent which factors are important and which are not, this needs to be determined by modelling and experimental validation.

A full model will incorporate multiple determinants of amino acid transport, for instance the different types of amino acid transporters and blood flow through the maternal and fetal circulations (Fig. 1). Before these determinants can be effectively combined into one model it is necessary to demonstrate that these factors can be modelled individually. We will begin by testing simple scenarios and, only when this is successful, move on to more complex sequence. We have previously reported on modelling the interaction of specific transporters with two amino acids [4]. This provides an example of the simple scenarios on which a fuller, more complex model could be based.

As the model develops, it should be able to make predictions that can be tested experimentally. Where the model is able to make experimentally verifiable predictions more complex scenarios will then be tested. Where the model is not able to make experimentally verifiable predictions this suggests that our assumptions need to be revisited. To develop a functional model, it will be necessary to have a clear understanding of the factors of likely importance. These are discussed below.

3. Membrane transport of amino acids

Amino acids are transported across the microvillous plasma membrane (MVM) and the basal plasma membrane (BM) of the placental syncytiotrophoblast (Fig. 1). Net transfer generally occurs in the maternal to fetal direction, against the concentration gradient, with the amino acid concentrations in fetal blood being significantly higher than those in maternal blood [5], so the process must utilise energy. Transport of amino acids across this exchange barrier involves multiple different membrane transport proteins, differentially localised to the MVM and BM [3] (Fig. 1). Amino acid transporters use three broad classes of transport mechanism: accumulative transport, amino acid exchange and facilitated transport [6]. These transporter classes fulfil different but interdependent roles and no one class is sufficient for mediating the transport of all the amino acids required by the fetus. Amino acids only diffuse very slowly across biological membranes and understanding the amino acid transporters must be central to any model.

Accumulative transporters mediate secondary active uptake of amino acids into the cell driven by electrochemical gradients across the plasma membrane. Accumulative transporters establish amino acid gradients within the syncytiotrophoblast that drive the activity of amino acid exchangers and facilitated transporters. As such they are important on the MVM of the syncytiotrophoblast where they mediate uptake of maternal amino acids. However, it is not clear what role accumulative transporters play on the BM where they will mediate uptake of fetal amino acids but not efflux to the fetus. The only accumulative transporter that does have a clear role on the BM is system X^{-}_{AG}, which mediates uptake of fetal glutamate into the placenta for metabolism.

Amino acid exchangers transport one amino acid across the plasma membrane in exchange for another. This has the effect of altering amino acid concentrations qualitatively without affecting the osmolality of the cell [7]. Exchangers play an important role on both the MVM and the BM and mediate the transfer of amino acids not transported by either accumulative transporters on the MVM or facilitated transporters on the BM. The activity of exchangers will be determined by amino acid concentrations on both sides of the plasma membrane. So the activity of other amino acid transporters and the delivery of substrate by blood flow, which both affect amino acid concentrations, are crucial to their function.

Facilitated transporters mediate bi-directional transport of amino acids, with net transport occurring in the direction of the concentration gradient. These transporters are thought to be primarily localised to the BM where they mediate net efflux of specific amino acids down the concentration gradient established and maintained by transporters on the MVM [8]. As facilitated transporters are dependent on the concentration gradient, their activity depends on the amino acid concentrations both in the placenta and in umbilical arterial blood.

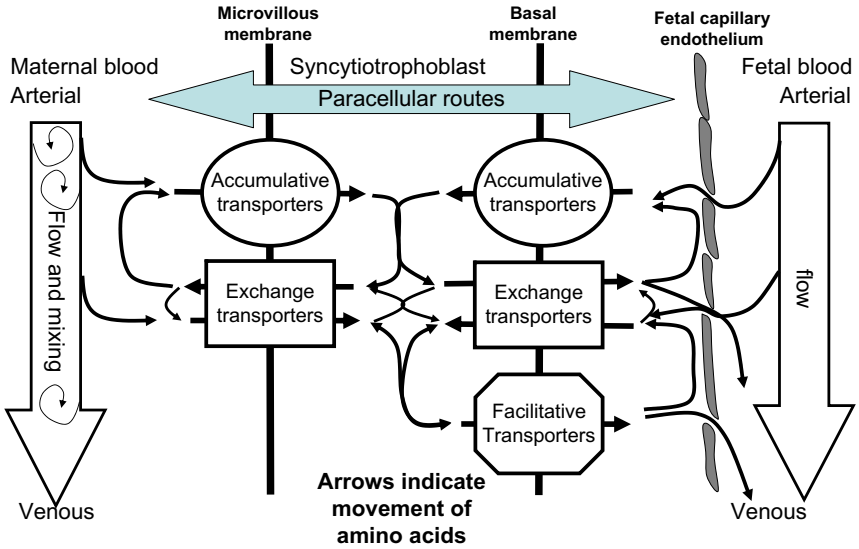

Fig. 1. Amino acid transfer across the human placenta involves complex interactions. Amino acids cross the syncytiotrophoblast via a range of transport proteins with different specificities and modes of action. Mathematically modelling these processes will explain how changing specific, individual components will affect the system as a whole.

So for amino acids to cross the placenta in the maternal to fetal direction, the MVM accumulative transporters and exchangers are required to mediate uptake from the maternal circulation and the BM facilitated transporters and exchangers are required to mediate efflux to the fetal circulation. Transporters on either the MVM or the BM may be rate-limiting [8]. The transport of particular amino acids cannot be considered in isolation as the transport of any one amino acid may affect that of others (Fig. 1). The effects on other amino acids is first due to competitive inhibition (a function of both concentration and K_M) and secondly due to the effect on amino acid concentrations on either side of the membrane.

In summary, transporter modelling needs to incorporate accumulative, exchange and facilitated transporters, with appropriate substrate specific affinities as well as relative activities. It must also be able to address the interactions between transporters and the way in which the actions of each transporter will alter the availability of amino acids for other transporters and hence their activity.

4. Placental structure

One of the major determinants of transporter activity is substrate concentration, both in the maternal and fetal circulations and within the syncytiotrophoblast. Placental structure and blood flow are important determinants of substrate concentrations at the site of transport and will therefore make an important contribution to transporter activity.

The normal human placenta is a fetal tissue which has a discoid shape and is 20–25 cm in diameter, 2–3 cm thick at its centre and weighing 400–500 g [9]. It is functionally divided into 10–40 units called cotyledons or lobules [9]. Each lobule has its own maternal blood supply and there is no mixing of maternal blood between lobules or between the fetal and maternal circulations [9]. Within each lobule maternal blood fills the intervillous space and directly bathes the fetal villous trees which are perfused by the fetal circulation.

The outer layer of the fetal villi is formed from a continuous syncytiotrophoblast that constitutes the primary barrier between the maternal and fetal circulations. Underlying the syncytiotrophoblast is a layer of cytotrophoblast which covers about 44% of the BM surface area at term [10]. The extent to which cytotrophoblast participate in nutrient exchange is unclear. The stromal interstitium between the syncytiotrophoblast BM and fetal capillary endothelium is filled with protein matrix as well as some cells including fibroblasts and macrophages. The final layer is the fetal capillary endothelium. Although endothelial cells do express amino acid transporters, it is likely that there is free diffusion of small water soluble nutrients, such as amino acids (74–204 Da), through capillary endothelial junctions. Free diffusion of small molecules is suggested by the ability of proteins to diffuse into this space (38800 Da) [11].

Three principle components of placental structure are likely to be key determinants of amino acid transfer and therefore require inclusion in the model. The first is size, as a larger placenta will generally have a greater surface area for nutrient exchange and so a greater transfer capacity. The second is the way in which placental structure affects blood flow and mixing in the two circulations as well as the relative alignment of the two systems which affects the efficiency of transfer. The human placenta is thought to have a multivillous system of maternal and fetal flow which allows for relatively effective nutrient transfer [12]. This influences the delivery of substrates to and from the transporting surfaces and thus the amino acid gradients which determine transporter activity. Thirdly, diffusion distances between the maternal and fetal circulations may be important [13].

Once across the BM, the rate at which amino acids are able to diffuse away will determine their gradients across the BM and thus exchanger and facilitated transfer across this membrane. This rate of diffusion between the external face of the BM and the fetal blood will be determined by the permeability of the stromal interstitium to amino acids, the junctions between endothelial cells and the endothelial glycocalyx [14].

Placental structure will be represented in our model by summary measures such as surface area of the exchange surfaces, volumes of the compartments, and capillary length [4]. This approach is favoured as it is considerably less complicated than modelling three dimensional placental structure in its complexity and summary measures can be determined experimentally using morphometric techniques to allow the validation of the model [15].

5. Maternal blood supply

The maternal uterine circulation delivers the amino acids that are transferred to the fetus by the placenta. Uterine blood flows through spiral arteries into the placental intervillous space where, as there is no maternal vasculature, it is in direct contact with fetal villi. After mixing within the intervillous space, maternal blood flows out through venous openings back into the uterine circulation. The localisation and number of spiral arteries and veins entering and leaving the placenta is not yet clear but will affect the flow and mixing of blood within the intervillous space [9]. It is likely that there are 30–40 spiral arteries per placenta, so one or two per lobule [16]. The number and localisation of uterine arteries and veins will determine blood flow within the lobule and clarification of this aspect of placental anatomy is of particular relevance to models of flow [17].

Blood entering the intervillous space must mix with the blood already present and the extent to which mixing occurs will affect the amino acid concentrations available for transport. It has been hypothesised that poor mixing, in circumstances such as preeclampsia, may impair transfer [16].

6. Fetal–placental blood supply

Fetal blood is delivered to the placenta via the umbilical arteries and nutrient enriched blood delivered back to the fetus by the umbilical vein. Fetal arteries and veins branch across the surface of the chorionic plate to, and from, each villous tree. Most placental lobules will have one villous tree while larger ones may have several [9].

Within the villous tree, stem and intermediate villi supply terminal villi which are thought to mediate the majority of exchange and form the greatest proportion of villi in the term placenta [15]. The extent to which the stem and intermediate villi participate in nutrient exchange is not clear but it is likely that such transfer would be less efficient, with longer diffusion distances.

7. Amino acid metabolism

The concentration gradients which determine the rate of transport of amino acids across MVM and the BM of the syncytiotrophoblast are affected by metabolism. While maternal diet provides the ultimate source of amino acids, the composition in maternal plasma will be determined by the mother's metabolism [18]. Similarly, amino acid concentrations in the umbilical arterial blood will be determined by fetal metabolism. Thus any change in maternal or fetal metabolic status may affect placental transport of amino acids.

Within the placenta there is inter-conversion of amino acids [19] and they are also utilised for protein synthesis, intermediary

metabolism, energy production and biosynthetic pathways. Interconversion of amino acids will alter the concentration gradients of two different amino acids, the one which is broken down and the one which is synthesised, while consumption will reduce the concentration of amino acids available for transfer. The effects of metabolism will particularly affect the activity of facilitated transporters and exchangers whose activity is directly determined by the concentration gradient.

Another metabolic influence on intracellular amino acid levels, which has not yet been well characterised, is protein turnover. Amino acids are both taken up into, and released from, the protein pool and at steady state uptake will equal release. A sufficiently high rate of protein turnover would buffer variations in maternal amino acid supply.

8. Paracellular routes

There is clear physiological evidence that small solutes, including amino acids, cross the placenta via a paracellular route [20,21]. The anatomical nature of this route is uncertain and paracellular transfer may either occur via regions of syncytial damage or via trans-syncytial channels [11,22]. Transfer of amino acids by the paracellular route could occur by simple diffusion and, if a pressure difference exists, by convection.

For both diffusion and convection, net transfer would be in the fetal to maternal direction. In the case of diffusion this is due to the fetal to maternal concentration gradient [5] and for convection the fetal to maternal pressure gradient [23].

The electrical potential difference across the human placenta (distinct from the transmembrane potential) could potentially drive paracellular flux of charged amino acids. However, at term the transmembrane potential is reported to be small and is unlikely to be a major driver of diffusion [24,25].

Transfer by paracellular routes needs be incorporated into the model using a diffusion coefficient based on experimental data for paracellular markers of similar molecular size [21]. Transfer by convection may also need to be considered but there are not currently good estimates of the magnitude of this phenomenon [23].

9. Our modelling approach

Of the factors discussed above the key elements that will be most important in the model broadly fall into two categories, the amino acid transporters (Fig. 1) and the factors that determine the concentrations of amino acids to which the transporters are exposed (Table 1).

Modelling the amino acid transporters together requires modelling the individual transporters themselves and to model how they interact. Since our initial models based on phenomenological Michaelis Menten kinetics [4], we have further developed our models to make them more mechanistic (Fig. 2). We use “carrier” type models, in which the amino acid needs to bind to a transporter in order to cross the membrane. These models are based on an abstract representation of the transporter in which a number of different states are distinguished to describe all possible conformational changes and binding configurations on each side of the membrane [26,27]. The transporter can then alternate between these states, as governed by binding and mobility parameters, which gives rise to the specific transporter behaviour. Mathematically this translates in a single equation for the net amino acid flux as a function of amino acid concentrations on each side of the membrane. We are testing these carrier models in the laboratory using MVM vesicles which allow for a highly controlled environment and for electrochemical gradients to be easily manipulated across the plasma membrane [28,29].

Table 1
Factors which affect amino acid concentrations in the maternal, syncytiotrophoblast and fetal compartments.

Factor	Effect on amino acid concentrations and transporter activity
Maternal metabolism	Determines uterine amino acid concentrations which affects concentrations in the intervillous space
Maternal spiral artery flow	Affects the rate at which arterial blood is delivered to the placenta and the rate at which amino acid depleted blood is removed
Volume and structure of the intervillous space	Determines how effectively amino acids from arterial blood will mix and reach the sites of transport on the MVM
MVM Transporters	Changes amino acid concentrations in the intervillous space and within the syncytiotrophoblast
Syncytiotrophoblast volume and surface area	Volume will determine the concentration change due to the influx or efflux of a given amount of amino acids. Surface area will constrain the number of transporters which can be expressed
Placental metabolism	Amino acid concentrations will be affected by catabolism, anabolism, inter-conversion and flux into and out of the placental protein pool
BM transporters	Change amino acid concentrations within the syncytiotrophoblast and in the fetal compartment
Volume of stoma	Determines the concentration change due to the delivery or removal of a given amount of amino acids
Diffusion through endothelial junctions	Affects the amino acid concentrations at the BM and flux into the fetal capillary
Fetal capillary volume	Will affect the rate blood flow, vascular resistance as well as the concentration of delivered amino acids
Fetal umbilical blood flow	Determines the rate of delivery of umbilical arterial blood and the rate at which transferred amino acids across the placenta are removed from the site of exchange
Fetal metabolism	Determines umbilical arterial amino acid concentrations which affect the concentrations at the BM

Using these individual transporter models, a placental transfer model can be developed in which the individual transporters work together in an integrated way. At this point, elements of placental structure begin to be incorporated into the transfer model as

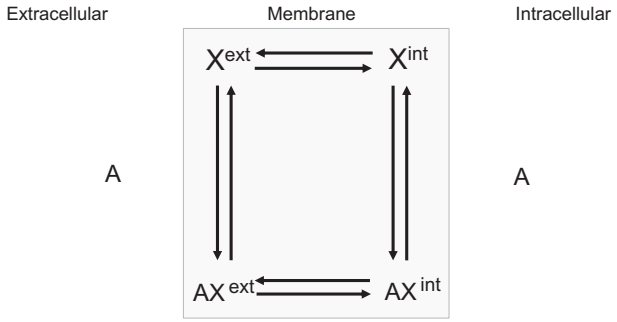

Fig. 2. Generic carrier model for amino acid transport. The amino acid is symbolized in the schematic by A while X represents the transporter/carrier and AX denotes the transporter-amino acid complex. X^{ext} is on the extracellular face of the membrane and X^{int} on the intracellular face. The arrows show the directions in which the fluxes can occur. As drawn, the carrier model represents a facilitated transporter. An amino acid exchanger is essentially the same except that the carrier can only ‘flip’ in the membrane if bound to an amino acid. To go through a complete cycle an exchanger must bind and transport an amino acid on one side of the membrane, and then an amino acid on the other side of the membrane. For Na^+ -dependent accumulative transporters the model differs in that the AX can only form if Na^+ also binds, the membrane potential and low intracellular Na^+ conferring the directionality of net transfer.

transporters must be located relative to placental compartments. We will test these models in the isolated perfused placenta, initially with simple scenarios and then with increasing complexity. First we will use a simple compartmental model, in which the maternal, syncytiotrophoblast and fetal compartments are characterised by their volume and surface area only [4]. The amino acid concentration in each compartment can then change due to transfer between compartments, which is governed by the transporter equations described above, as well as in and out flows (Fig. 3).

While the compartments are initially assumed to be well mixed, as a next step, modelling the factors which determine amino acid concentrations will involve analysis of blood flow, mixing, convection and diffusion of substrates through the maternal, syncytiotrophoblast and fetal compartments. Here we will be guided by established work on blood flow through the intervillous space, modelled as a porous medium and integrate this with models for the fetal vascular and capillary networks [17]. By conducting placental perfusion experiments, and then conducting morophometric analysis on the perfused lobules, we will test the validity of these flow models.

With any model a sensitivity analysis is essential to assess the impact of measured parameters and the associated uncertainty on model predictions. This can be accomplished by varying each of the model parameters around the physiological level. The results of this analysis will then indicate whether the uncertainty in specific parameters can either be safely ignored or whether efforts should be concentrated on measuring these parameters more precisely. In addition this will also reveal which parameters are the most critical determinants of transport within the model (Fig. 3).

Through a process of testing and retesting, we will identify which of the placental parameters tested are important for the model. When testing the model in a normal placenta it is important to remember that a factor that is not rate limiting may become so in a pathological pregnancy and vice versa. Once we have a full model, it will be tested using highly characterised pregnancies to determine conformity to the predictive model. In these pregnancies we will measure as many relevant parameters as possible including umbilical arterial and venous plasma amino acid concentrations and blood flows. All factors will be included in the model, except umbilical venous amino acid concentration and we will determine how effectively we can predict these through application of the functional model. The model that we are currently developing is based on parameters in term human placenta. This model would not be expected to predict function at earlier points in gestation where input parameters are likely to be different. However we intend that the model framework should be applicable to earlier points in gestation if the required input parameters (e.g. structure, transporter expression and localisation) became available.

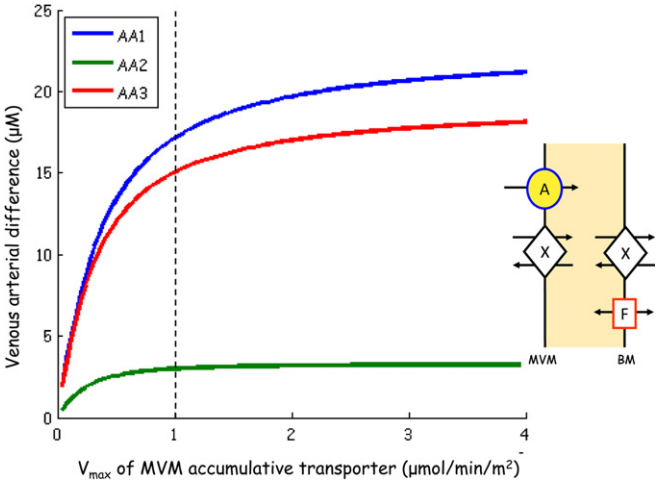

Fig. 3. The effect of increasing the V_{max} of the MVM accumulative transporter (A) on amino acid transfer to the fetal circulation in a simple model of placental amino acid transfer. Increasing V_{max} of the accumulative transporter (A) has a significant effect below the V_{max} of the other transporters (the exchanger (X) and facilitated transporter (F)) which is indicated by the vertical dashed line. However, above the V_{max} of the other transporters the effect of increasing the accumulative transport V_{max} diminishes as the other transporters becomes limiting. In this model there are three amino acids (AA; 1, 2 and 3) and three amino acid transporters, an accumulative transporter (A) localised to the MVM which only transports AA1, an exchanger localised to both the MVM and BM which transports all three amino acids (AA1, AA2 and AA3) and a facilitated transporter localised to the BM which only transports AA3. Transporter modelling was based on carrier-mediated transporter models. For the simulation above, blood flow was set as equal in the two circulations and arterial concentration of all 3 amino acids was 100 μmol/l in the maternal circulation and 150 μmol/l in the fetal circulation. All K_M values are 100 μmol/l and the activity of the exchanger (V_{max} μmol/min/m^2 surface area) and facilitated transporter are equal. The relative volumes of the three compartments are based on those determined by morphometry [15].

10. Implications

Modelling has significant potential to improve our understanding of placental amino acid transfer. Initially this will come from the process of using the model to test our assumptions about the processes involved. Then, once a functional model is established, it will allow investigation of how the determinants of transport interact and provide the basis for an integrated systems biology understanding of placental amino acid transfer. It will allow us to determine how each factor contributes to the process as a whole and identify those factors which influence predictive power of the model. This will be important, as it will help us to predict which factors are rate limiting and to focus future research on those factors which are most likely to cause fetal growth abnormalities such as fetal growth restriction (FGR) or fetal macrosomia.

There is currently no treatment for FGR other than iatrogenic early delivery with attendant risks for both mother and baby. The aim of developing a computational model of placental amino acid transport is to be able to understand abnormalities in FGR pregnancies and other pregnancy pathologies. This may allow rapid modelling of potential therapeutic strategies, prior to complex and expensive testing in animal models or in human pregnancy.

Conflict of Interest

The authors have no conflicts of interest to declare.

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